Progress in Biophysics and Molecular Biology 115 (2014) 115–128

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

journal homepage: www.elsevier.com/locate/pbiomolbio

Review

The cardiac muscle duplex as a method to study myocardial heterogeneity

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ARTICLE INFO

Article history:
Available online 5 August 2014

Keywords:
Cardiomyocyte interaction
Excitation-contraction coupling
Cardiac mechano-electric coupling
Electro-mechanical mathematical model
Cardiac tissue
Mechano-electric feedback

ABSTRACT

This paper reviews the development and application of paired muscle preparations, called duplex, for the investigation of mechanisms and consequences of intra-myocardial electro-mechanical heterogeneity. We illustrate the utility of the underlying combined experimental and computational approach for conceptual development and integration of basic science insight with clinically relevant settings, using previously published and new data. Directions for further study are identified.

Contents

List of abbreviations: 1D, One-dimensional; AP, Action potential; APD, Action potential duration; APD_{80}, AP duration at 80% repolarization; ΔAPD, Difference in APD; BM, Biological muscle; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; Ca–TnC, Ca^{2+} bound to TnC; CRT, Cardiac resynchronization therapy; DR, Dispersion of repolarization; ENDO, Sub-endocardial; EO model, Ekaterinburg-Oxford model; EPI, Sub-epicardial; F–V, Force–velocity relationship; LV, Left ventricle; L_{MAX}, Muscle length at which maximum isometric peak force is developed; ML, Initial muscle length; MP, Membrane potential; N-cell, Normal cell; SC-cell, Sub-critical cell; SR, Sarcoplasmic reticulum; SFR_{IM}, Intra-myocardial slow force response; TnC, regulatory protein troponin C; VM, Virtual muscle.

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http://dx.doi.org/10.1016/j.pbiomolbio.2014.07.010

1. Introduction

Cardiac pump function builds on, and requires, the interaction of regional spatio-temporal heterogeneities, from differences in electrical activation timing and gradients in load-dependent local stress-strain behaviour, to variation in passive and active cellular electro-mechanical properties (Katz and Katz, 1989). Indeed, cells isolated from various parts of the left ventricular (LV) wall have different ion handling protein densities (Antzelevitch and Fish, 2001; Wolk et al., 1999) and mechanical properties (Bollensdorff et al., 2011; Cazorla and Lacampagne, 2011; Cordeiro et al., 2004; Wan et al., 2003). Contractile protein isoforms differ (Litten et al., 1985; Stelzer et al., 2008), with V3 myosin dominating in the sub-endocardial layers, while in sub-epicardial myocytes the V1 isoform is more common in many species. These myosin isoforms differ in the dynamics of cross-bridge cycling, where V1 allows for greater velocity of cardiomyocyte shortening compared to V3 (Litten et al., 1985; VanBuren et al., 1995). Also, owing to differences in the expression levels of several ionic mechanisms, cells from sub-endocardial and sub-epicardial layers further differ in action potential (AP) shape and duration (Bryant et al., 1997; Stones et al., 2008), as well as in the kinetics of intracellular Ca^{2+} handling (Cordeiro et al., 2004; Laurita et al., 2003). As a result, together with faster contraction dynamics, sub-epicardial cardiomyocytes also demonstrate shorter AP and swifter Ca^{2+} transients than the (physiologically earlier-activated) sub-endocardial cells.

At the tissue level, structure and function of the LV have been shown to be highly heterogeneous (Ashikaga et al., 2007, 2009; Bogaert and Rademakers, 2001; Sengupta et al., 2006b). Regional mechanical function in the LV varies longitudinally from basal to apical segments, in the transmural direction and between the free wall and the septum (Ashikaga et al., 2009; Sengupta et al., 2006a). The timing of electrical excitation in the LV wall is also known to be heterogeneous, with excitation generally spreading from apex to base and from sub-endocardial to sub-epicardial layers (Sengupta et al., 2006b).

Despite a large body of research characterising cardiac regional heterogeneity, its role in the mechanical and electrical function of the myocardium, and the bilateral relationships between electrical and mechanical activity, remain under-appreciated.

Significant progress in understanding the role of heterogeneity phenomena has been achieved using simple models of heterogeneous myocardium, called "muscle duplexes", first developed in the 1960s by Tyberg and co-workers (Tyberg et al., 1969), and later expanded by Bing and colleagues (Shimizu et al., 1996; Wiegner et al., 1978). Tyberg's team studied mechanical consequences of interactions between end-to-end ('*in series*') connected normal and ischaemic muscles. Bing's group added a computer interface to record muscle contraction in normal conditions, and then to apply this as a command signal to the same muscle after exposure to hypoxia. The authors interpreted this kind of signal exchange as *in series* interactions between the muscles, although the signal from the setup was in fact uni-directionally applied to the biological sample.

The cardiac muscle duplex approach was subsequently developed further, and specifically applied to investigations into cardiac regional heterogeneity, by our group (Markhasin et al., 1999; Rutkevich et al., 1997). A muscle duplex represents the simplest physiological model of mechanically interacting segments of myocardium. It comprises two isolated and mechanically coupled muscle elements, exposed to *bilateral* mechanical interactions during their contractions. As this system is simple, it allows one to unravel basic properties of heterogeneous myocardium, as they emerge from element interactions. We have refined the duplex method by implementing a number of principally different configurations, including *in series* and *in parallel* coupling of biological and virtual (computer-modelled) muscles (Markhasin et al., 2003), and by extending its capability by simultaneous registration of cellular electrical activity or Ca^{2+} dynamics (Markhasin et al., 2012). This allowed us to reveal and explain a number of basic effects characteristic of heterogeneous myocardium in norm and pathology.

Here, we review the duplex techniques and illustrate essential results obtained with our approach. Most of these results have been published in previous papers, and the relevant sources are identified in the text.

2. Muscle duplex approach

To address the effects of mechanical interaction between spatially distinct but mechanically coupled segments of native myocardial tissue we use the simplest case model – the muscle duplex (Markhasin et al., 2003). Muscle segments are mechanically connected either *in series* or *in parallel* and different sequences of muscle stimulation with varying time lags (from 0 to ±100 ms) are applied to simulate time delays between regional excitation throughout the myocardial tissue. The physiological relevance of the duplex model stems from the fact that mechanical signal transduction in cardiac tissue is more far-reaching, and two to three orders of magnitude faster, than electrical excitation propagation: mechanical stimuli travel near the velocity of sound in liquids, i.e. about 3×10^2 m/s, compared to electrical conduction speeds in the order of 10^{-1} to 10^0 m/s. Mechanical effects from earlier activated myocardial segments are therefore almost immediately transmitted even to distant surrounding tissue, potentially affecting its subsequent activity via mechano-mechanical (Shiels and White, 2008), mechano-electric (Kohl et al., 1999), mechano-chemical (Ennis et al., 2013) and mechano-structural feedback (Kohl et al., 2003).

We developed and explored six principal duplex configurations (Markhasin et al., 2003; Protsenko et al., 2005), using either *in series* or *in parallel* mechanical connections between coupled muscles, implemented for three sets of element combinations: (1) a biological duplex comprising two isolated multicellular myocardial preparations (biological muscles [BM]; i.e. thin papillary muscles or trabeculae); (2) a virtual duplex comprising two computational models of the electro-mechanical activity of cardiac muscle (virtual muscles [VM]; see below for details); or (3) a hybrid duplex comprising one BM and one VM. A schematic illustration of all the

duplex settings is presented in the electronic supplemental data (see Fig. S1).

2.1. Main features of mechanical interactions between in series and in parallel coupled muscles

In the *in parallel* duplex, dynamic interactions of elements occur at identical lengths, for example during shortening-lengthening phases of isotonic or auxotonic contractions of the pair, working from (against) a defined and externally applied mechanical pre- or afterload. Here, element forces add up to total duplex force, while element deformations are equal at any given time (see Fig. 1 and Fig. 2, left panel). This kind of dynamic behaviour of coupled muscle segments mirrors certain aspects of the interactions between *in parallel* ventricular layers (e.g. sub-endocardial and sub-epicardial regions), where individual regional forces are in balance with the external mechanical load during overall chamber deformation (Ashikaga et al., 2007; Sengupta et al., 2006a).

The *in series* duplexes can be used to investigate dynamic interactions between 'end-to-end' coupled muscles, as they occur during externally isometric contractions. When imposing externally isometric conditions on the whole duplex, the outer ends of muscles are kept iso-positional, while internal changes in element lengths are allowed and registered (see Fig. 2, right panel, and Fig. 3 showing muscle interactions). Mechanical activity of these *in series* duplex elements is governed by coinciding yet opposite length changes, as they 'pull' at each other, while forces in the elements are equal at any given time. Therefore, each duplex element contracts *auxotonically*, as is the case in real tissue. The ensuing dynamic behaviour of coupled muscle elements is conceptually similar to isovolumetric contraction (or relaxation) of the ventricles, where internal regional deformations occur at constant ventricular volume (Ashikaga et al., 2009; Sengupta et al., 2006a).

2.2. Main features of the biological, virtual and hybrid duplex settings

2.2.1. Biological duplexes

These consist of two mechanically linked BM that are kept in separate chambers with independent stimulation, perfusion and temperature control systems. Mechanical interaction is implemented using a computer-controlled system to interrelate input/output signals of muscle force and length changes between both BM, with an internal time step of 100 μs, while prescribing the relevant duplex constraints (i.e. in externally isometric *in series* duplexes – opposite length changes and equal force, or for contracting *in parallel* duplexes – opposite force changes at equal shortening). The principal features of the experimental duplex method and a schematic illustration of the computer-based control algorithm used to imitate real-time mechanical interactions are available elsewhere (Markhasin et al., 2003; Protsenko et al., 2005). In addition to the mechanical activity of both BM, the duplex setting allows us to measure action potential (AP) time courses using floating microelectrodes (see an example of AP recordings in Section 3.3, and (Markhasin et al., 2012)). Unfortunately, due to the dynamic nature of the preparation, it has been difficult to measure AP in both BM simultaneously and for prolonged periods, even with 'floating' microelectrodes, in particular when switching between freely-contracting BM elements and coupled duplex.

2.2.2. Hybrid duplexes

A similar control algorithm is used to implement muscle interactions in hybrid duplexes, where computer model calculations are synchronized with the signal exchange between BM and VM (the latter implemented as a computer model-driven mechanical actuator) in real time (Protsenko et al., 2005). One of the main benefits of the hybrid duplex configuration is that it allows one to expose the same BM to interactions with various VM (e.g. by simulating mechanical coupling of a BM to a 'slow' or a 'fast' VM, as described below). This can be utilized to simulate a wider range of functional heterogeneities than is usually possible in the biological duplex setting. In VM, we can further analyse model parameters, including muscle force and sarcomere length, AP, intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), regulatory Ca^{2+} complex behaviour, sarcoplasmic reticulum (SR) loading, or any other parameter contained in the model. In the BM, mechanical activity, AP, or Ca^{2+} transients (monitored with fura-2) can be recorded (see examples of Ca^{2+} transient recordings in (Markhasin et al., 2012)).

2.2.3. Virtual duplexes

Virtual duplexes comprise two coupled VM, each represented by an "Ekaterinburg-Oxford" (EO) model of cardiac electro-mechanical activity, linking the Oxford ionic model with the Ekaterinburg descriptions of Ca^{2+} handling and mechanical activity in ventricular myocardium (Solovyova et al., 2003). A CellML model representation of the EO model is available for download at the CellML

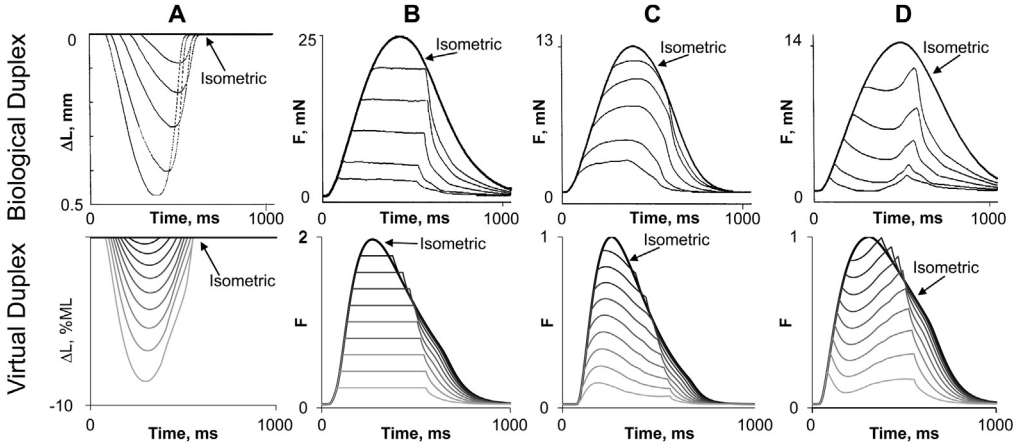

Fig. 1. Afterloaded contractions of *in parallel* duplexes. Top: experimental recordings of the mechanical activity in a biological duplex composed of two thin papillary muscles from rabbit right ventricle. Bottom: results of numerical experiments in a virtual duplex. Time courses of duplex shortening (column A), duplex force (column B) and force of each muscle element (columns C–D) at different afterloads. Note use of normalized y-scales for VM (ΔL normalized to the initial muscle length (ML)); F normalized to single element isometric peak force). Experimental data are from Solovyova et al. (2002), with permission.

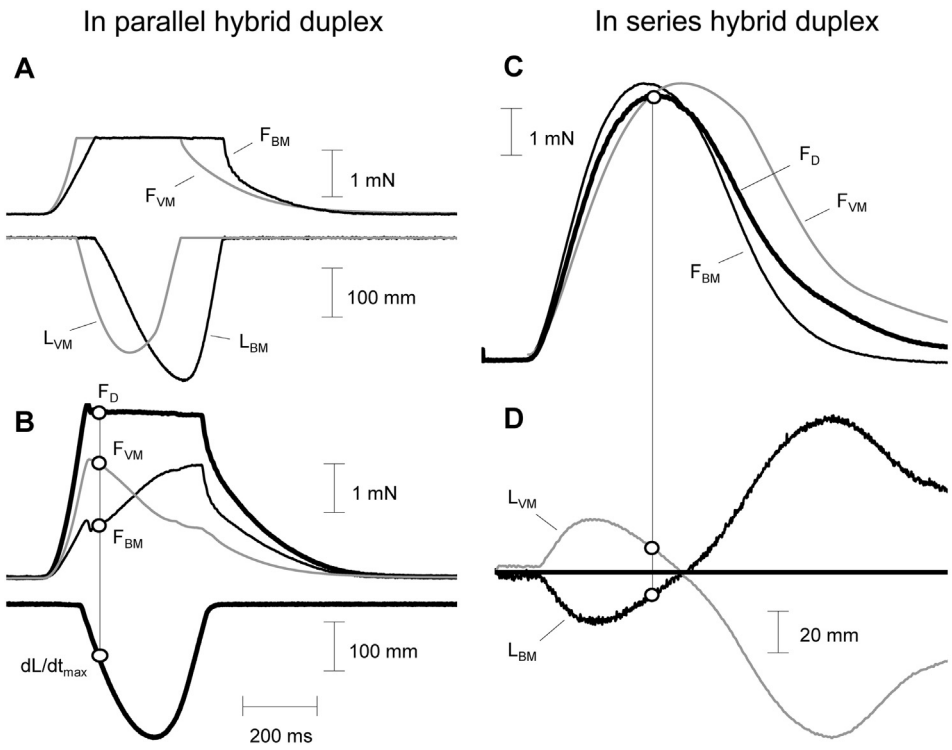

Fig. 2. Experimental recordings of force development and shortening of an *in parallel* (A and B) and an *in series* (C and D) hybrid duplex. A: force and shortening of a rat papillary biological muscle (F_{BM}, L_{BM}) and a virtual muscle (F_{VM}, L_{VM}) during afterloaded contractions in isolation. B: forces of duplex (F_d) and elements after *in parallel* connection, and overall duplex shortening. C: forces of the same muscles as in A, contracting in isolation (thin lines), and after formation of an *in series* duplex (thick line) during isometric contraction. D: length changes of these *in series* duplex elements, during externally isometric contraction. Vertical lines are drawn through point of maximal duplex rate of shortening (B) and maximal duplex force production (C, D), to highlight dynamics in ensemble behaviour at characteristic points of duplex contractions. From Protsenko et al. (2005), with permission.

model repository (http://models.cellml.org/e/b9/); it can be run using Cellular Open Resource tools (available for example at http://cor.physiol.ox.ac.uk/(Garny et al., 2003, 2009)). A key feature of the EO model is inclusion of the cooperative dependence of thin filament Ca^{2+} activation, particularly the relations between the concentrations of attached crossbridges and Ca^{2+} complexes with the regulatory protein troponin C (TnC). We have previously demonstrated that the crossbridge-induced increase in the affinity of TnC for Ca^{2+} is a key mechanism and vital contributor to the effects of cardiac excitation-contraction coupling and mechano-electric feedback (Solovyova et al., 2003; Sulman et al., 2008).

To simulate the effects of VM interactions in heterogeneous ventricular tissue, where cardiomyocytes from distant regions differ in their electro-mechanical properties (e.g. transmural or base-apex gradients; reviewed in (Markhasin et al., 2011)) we developed so called fast and slow VM. These VM differ in their velocity of contraction and relaxation, AP duration, and the rate constants of intracellular Ca^{2+} kinetics (for detail, see (Solovyova et al., 2003)). Superposition of afterloaded contractions of fast and slow VM samples at different afterloads are shown in the online supplement (Fig. S2).

For modelling VM properties, one can use any available model of cellular electro-mechanics, such as developed by several groups (e.g. (Campbell et al., 2008; Gurev et al., 2010; Matsuoka et al., 2003; Niederer et al., 2009; Rice et al., 2008)). These models tend to differ in various aspects of cardiac electrophysiology, Ca^{2+} kinetics, and myofilament mechanics, as well as in their approach to simulating excitation-contraction coupling and, crucially for duplex investigations, mechano-electric feedback (for comprehensive reviews, see (Henriquez, 2014; Trayanova and Rice, 2011; Trayanova et al., 2011), as well as the CellML repository (http://models.cellml.org/cellml)). Application of different 'cell models' would affect VM behaviour, and a comparison of duplex models based on the different models, as well as their validation against data obtained in duplexes using BM, would be an interesting approach towards unravelling model-specific *vs*. generalizable features of intra-myocardial interactions, using an experimental setting (with high analytical utility) for which the individual cell models 'have not been trained'.

2.3. Temporal heterogeneity of muscle element activity in duplexes

Here, and in most of our previous work, we address effects of myocardial mechanical interactions that result mainly from temporal heterogeneities in the mechanical activity of coupled muscle elements. This arises as a consequence of two factors: (1) the presence of a time-lag in muscle electrical activation (electrical asynchrony), and (2) heterogeneity of muscle force development/shortening dynamics (mechanical asynchrony). Accordingly, three forms of internal heterogeneity of muscle duplexes were considered: electrical asynchrony, mechanical asynchrony, and a mix of both.

If in biological duplexes two BM are similar, in terms of their contraction-relaxation kinetics, temporal heterogeneity can be introduced via a stimulation delay between the two BM. Mechanical heterogeneity can be induced using biological manipulations, such as different temperatures in the experimental bath chambers (warm BM contract faster, but generate lower peak forces). In hybrid duplexes, muscle heterogeneity can be modified by imposing stimulation delays, and via simulation of VM with different mechanical properties. In virtual duplexes, interactions of one fast and one slow VM were systematically explored during different stimulation delays to enhance or decrease temporal heterogeneity.

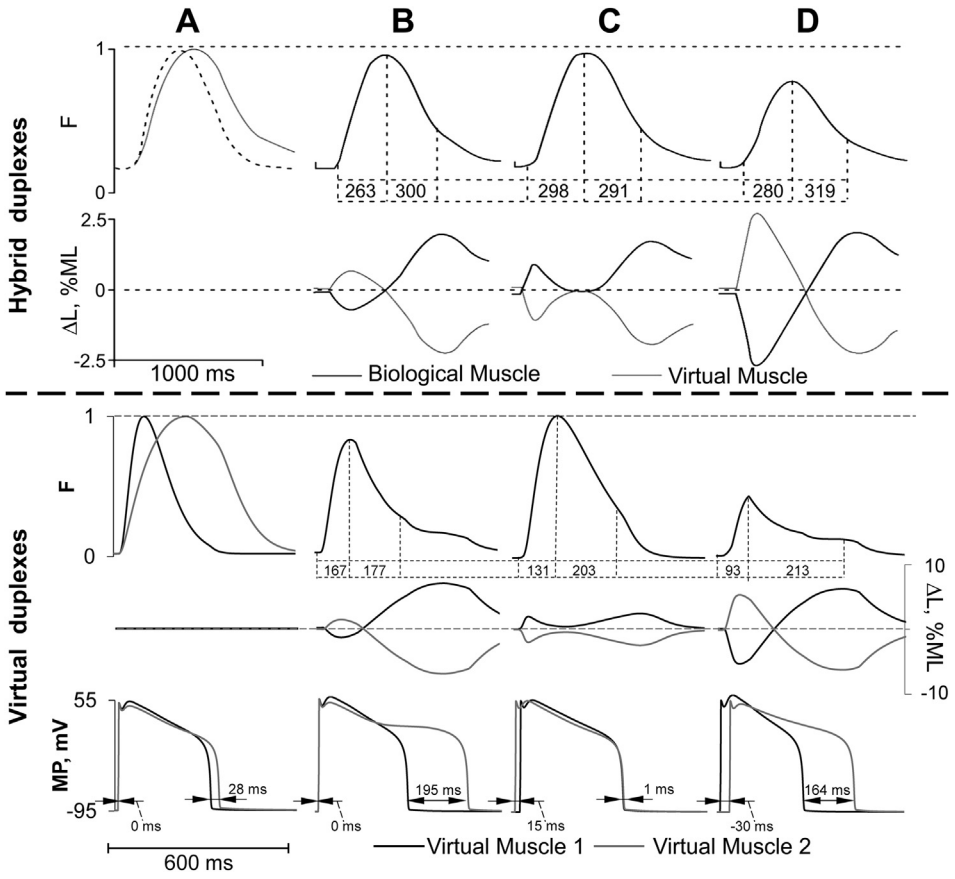

Fig. 3. Effects of stimulation delay on externally isometric contractions of *in series* hybrid (*top*) and virtual (*bottom*) duplexes. The hybrid duplex is composed of a fast BM (papillary muscle from rat right ventricle, stimulated at 0.3 Hz, at 30 °C, dashed line) and a slow VM (solid line). Duplex force (F) and muscle shortening (ΔL normalized to ML) are shown at various stimulation delays of either element. Isometric force, developed by muscle elements in isolation, is shown in panel A. From left to right: effects of simultaneous stimulation (B), and 60 ms delay of either the fast muscle (C) or the slow muscle (D). Time-to-peak force and time to 30% relaxation are indicated in the middle row. In the virtual duplex, membrane potential (MP) and corresponding dispersion of repolarisation (DR) are demonstrated, along with mechanical activity. Experimental data are from (Markhasin and Solovyova, 2005), with permission.

We usually excluded additional heterogeneity factors, such as differences in baseline peak force (i.e. weak *vs*. strong) or initial lengths (short *vs*. long) of duplex elements. In virtual or hybrid duplexes, we defined the VM to have the same initial lengths and baseline peak forces as the other duplex element (whether VM or BM). In experiments with two BM, this restriction was implemented using relative length and force amplifying coefficients when transferring mechanical outputs between the individual elements. This involved applying command input/output signals that simulated matching lengths and peak forces, e.g. if one BM shortened by 1% of its individual L_{MAX}, the second muscle was stretched by 1% of its own L_{MAX} (L_{MAX} is defined as the muscle length at which maximum isometric peak force is developed). This allowed us to unify initial conditions for muscle coupling, and to compare results obtained across different duplex studies.

2.4. Experimental protocols

To explore the effects of mechanical interaction between duplex elements, we use the following approach. First, we allow each of the two duplex elements to contract in isolation in isometric mode, at a length of 0.9–0.95 L_{MAX}, until steady-state contractions are reached. Parameters recorded (muscle force and length, AP or Ca^{2+} transient if available in BM; all model variables in VM) in this setting serve as the baseline to compare values after switching the mode of contraction (e.g. from isometric to isotonic mode, or after duplex formation). Then, muscles are mechanically interconnected, either *in parallel* or *in series*, and effects of their interactions are monitored. In experiments where slowly developing effects of interactions are monitored, we record the activity in interacting elements before and during the approach to a new steady-state in the duplex. Then, duplex elements are disconnected and returned to initial conditions, to assess presence of sustained changes in element contractile state.

3. Effects of mechanical interactions in heterogeneous duplexes

In all duplex settings (biological, virtual, and hybrid), mechanical coupling of two cardiac muscles causes significant changes in their mechanical behaviour (Markhasin et al., 2003; Protsenko et al., 2005; Solovyova et al., 2002, 2003). Moreover, as shown in VM and in those BM where either electrical activity or Ca^{2+} kinetics were recorded, mechanical interactions between segments cause modulation of cellular Ca^{2+} handling, and AP generation in both muscles (Markhasin et al., 2003, 2012; Protsenko et al., 2005; Solovyova et al., 2002, 2003). Individual responses depended on the sequence of muscle activation, imitating variations in the delay and direction of the excitation spread in the tissue.

3.1. Electro-mechanical effects in the in parallel muscle duplexes

In afterloaded *in parallel* mode of duplex contractions, duplex shortening starts when the sum of element isometric forces

approaches and exceeds the afterload level. As the timing of individual force development can differ between elements (due to electrical activation differences and/or mechanical asynchrony), element contributions to the overall duplex force vary during the joint shortening-lengthening cycle. Therefore, both elements contract auxotonically, and with different and dynamically changing loads (Figs. 1 and 2, left). When a duplex is formed of elements with similar peak isometric forces and isotonic shortening dynamics, duplex peak shortening is also similar to that of each element. If duplex elements are distinct in the velocities of isometric force/isotonic shortening, duplex shortening occurs with significantly differing dynamics for each of the two elements. The force–velocity dependence (a plot of the peak velocity of shortening during contraction at a given relative afterload against the load value), registered in coupled muscles, is then between the curves for the elements obtained in isolation (Fig. 4). Effects, similar to those predicted in virtual duplexes, were observed in experiments on biological (Fig. 1) and hybrid duplexes (Fig. 2; (Markhasin et al., 2004; Solovyova et al., 2002)). Unexpectedly, in all configurations of *in parallel* duplexes, individual force–velocity relations for muscle segments change significantly, and in opposite directions, during their interaction in the duplex, as revealed by comparison of individual element characteristics during mechanical coupling with values obtained before duplex formation (see Fig. 4, and (Markhasin et al., 1999; Protsenko et al., 2005; Solovyova et al., 2002)).

Both in models and experiments, we found that convergence or divergence of the force–velocity dependencies is determined by the activation sequence of duplex elements. A delayed electrical activation of the faster element of *in parallel* duplexes directs individual force–velocity curves towards each other, up to coincidence with that of the whole duplex at optimal activation delays between elements (Fig. 4, top). In contrast, in the case of simultaneous or delayed activation of the slower element, the force–velocity curves of elements move apart from one another, even beyond differences observed in the individual elements in isolation (Fig. 4, bottom).

These changes in mechanical activity result in opposite changes in the useful work, performed by each of the coupled muscle elements, during duplex shortening (as compared to work done in isolation; see Fig. S3 in the supplement and (Markhasin et al., 1999, 2004)). Convergence of the force–velocity curves coincides with a noticeable convergence of the dependencies of useful work on the afterload, making element performance more concordant within the duplex at any given afterload. In contrast, divergence in force–velocity curves is accompanied with a divergence in useful work, produced by the coupled elements during contraction, making individual muscles more heterogeneous than before coupling. The same effects were found both in models and in experiments (Fig. S3, and (Markhasin et al., 1999, 2004)), and they may point to some of the mechanisms underlying success or failure of cardiac resynchronization therapy.

Model analysis allowed us to reveal the mechanisms underlying the mechanical phenomena observed in muscle duplexes. We found that the reciprocal changes in force–velocity dependencies of muscle elements are caused by a dynamic redistribution of mechanical loads between coupled muscle elements during contraction. This gives rise to modulation of mechano-dependent Ca^{2+}-activation of myofilaments, as revealed by opposite changes in the kinetics of Ca^{2+} bound to TnC (Ca–TnC) and the number of force-generating cross-bridges in cardiomyocytes of VM (see Fig. S4 in the supplement and (Solovyova et al., 2002)). In the first-activated duplex element, the force–velocity dependence moves up in the duplex, while in second-activated muscle it decreases. Thus, one duplex element 'gains' contractility at the expense of the other, due to element mechanical interaction, and in the absence of direct exchange of matter (no paracrine activation, no inter-element transport of ions, etc.).

The changes in myofilament activity of interacting VM and their altered Ca–TnC kinetics affect cytosolic Ca^{2+} levels, which in turn can modulate other Ca^{2+}-dependent currents and affect AP properties. These AP changes are also opposite in direction in the cells of each muscle (see Fig. S4 in the supplement, and (Solovyova et al., 2002)). In heterogeneous duplexes, activation delays of the fast muscle (which has a shorter AP duration; APD) reduces the dispersion of repolarization (DR) up to total elimination at optimal stimulation delays (numerically, this delay is close to the difference in APD (ΔAPD) in the uncoupled muscles, as shown in VM in Fig. S4). Moreover, the repolarization sequence may even reverse direction, compared to the activation sequence, and the thus emerging duplex behaviour is consistent with the regional excitation-relaxation timing in the intact ventricle. In contrast, in duplexes with inverted activation sequence (fast first, slow second), DR increases, rendering them 'non-physiological', but at the same

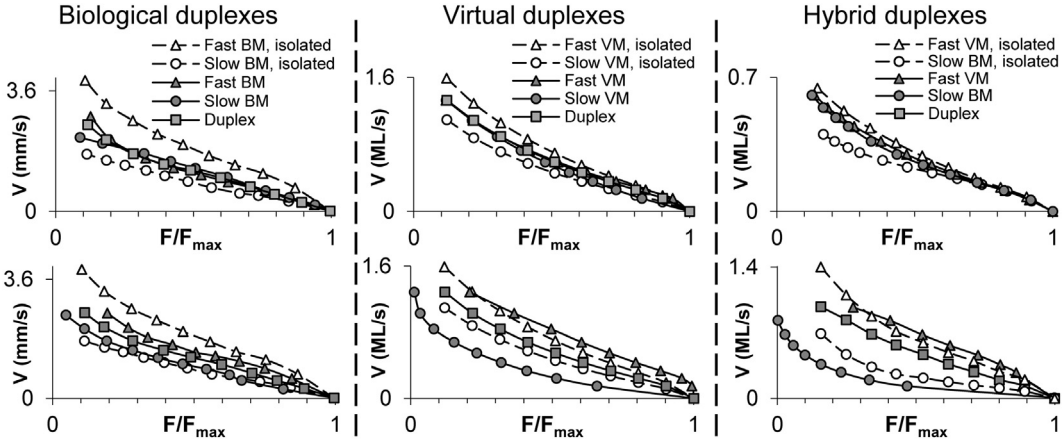

Fig. 4. Effects of excitation sequence in biological (left), virtual (centre), and hybrid (right) *in parallel* duplexes on the force–velocity relationships (F–V). Shown are the F–V dependencies obtained for a fast and a slow muscle element either in isolation (dashed lines) or when interacting within the duplex (solid lines), and for the duplex as a whole (solid lines with square symbols). F–V curves are obtained by plotting the maximum velocity of shortening *vs*. the force developed at the time when peak velocity is reached. Duplex force is normalized by its maximum (i.e. isometric peak force), whereas the muscle element forces are normalized by their own isometric peaks. Top row shows duplexes with a delay in fast muscle activation, bottom rows show a biological duplex with simultaneous element stimulation, and virtual and hybrid pairs with a delay of stimulation of the slow element. Experimental data are from (Protsenko et al., 2005; Solovyova et al., 2002) with permission.

time offering a suitable model for studying a setting similar to epicardial pacing, such as used for LV resynchronization approaches.

3.2. Electro-mechanical effects in the in series muscle duplexes

Figs. 2C–D and 3 show examples of isometric contraction of *in series* duplexes in different configurations. The main feature of externally isometric *in series* duplexes is that end-to-end coupling of individual muscle elements causes them to switch from isometric to auxotonic contractions, governed by opposite length changes in the elements (see Figs. 2D and 3, and (Markhasin et al., 2003; Protsenko et al., 2005; Solovyova et al., 2002, 2003)).

In heterogeneous *in series* duplexes of all configurations, force development strongly depends on activation sequence and time delay between element stimulation. Delayed stimulation of the fast element over a wide range of delays (from 0 to 100 ms) does not lead to major changes in the duplex peak force (see Fig. 3, and (Solovyova et al., 2003)). In contrast, *in series* duplexes are very sensitive to delayed activation of the slow element. This gives rise to pronounced negative effects on duplex peak force and contraction-relaxation dynamics (Fig. 3, and (Markhasin et al., 2003)). Comparable effects of stimulation delay on duplex peak force, depending on element activation sequence in duplexes of strong and weak elements, were shown in (Tyberg et al., 1969).

Similar to *in parallel* duplexes, cyclic dynamical deformations of VM elements, caused by their mechanical interactions, affect cellular Ca^{2+} kinetics and AP shape and duration in each element. The DR in virtual duplexes decreases if fast element stimulation is delayed (slow first, fast second), while the inverse of this activation sequence causes a pronounced increase in DR (see Fig. 3, and (Solovyova et al., 2003)). In homogeneous duplexes, composed of near-identical muscle elements, delays between element activation always causes a reduction in peak mechanical performance and an increase in DR (Solovyova et al., 2003), though this is less pronounced compared to late activation of slow muscle.

3.3. Intra-myocardial slow force response

As shown above, mechanical interactions between duplex elements causes significant changes in their functional state. Functional adjustment of elements to their new mechanical conditions (after duplex formation) occurs over a number of beats (from tens to hundreds), until a new steady state is reached (Markhasin et al., 2012; Solovyova et al., 2006). These slow changes in the total duplex force development were found in all duplex settings (Fig. 5, phase 2–3 in the force transition). Thinking of duplex elements as mechanically interacting regions of heterogeneous myocardium, and in view of the time course of their response to duplex formation, it is tempting to consider these arguably mechanically-induced changes in contractile activity as an equivalent of the so-called slow force response (Kentish and Wrzosek, 1998). The slow force response describes a gradual and continued (over tens to hundreds of beats) change in peak force, following a step-change in preload, the initial response to which is referred to as the Frank-Starling effect (Shiels and White, 2008). While the Frank-Starling effect (stretch-induced increase in contractility) is instantaneous and generally assumed to not be associated with measurable changes in intracellular Ca^{2+} transients, the slow force response is a result of altered cellular Ca^{2+} balance.

To highlight similarities (changes in effective load affecting contractility) and differences (dynamic nature of mechanical loading), and to refer to what we believe to be the physiologically relevant context, we call this gradual response to muscle interactions an 'intra-myocardial slow force response' (SFR_{IM}) (Fig. 5, and (Markhasin et al., 2012)). Qualitatively similar manifestations of SFR_{IM}, observed in BM of different animal species (rat, rabbit, guinea pig) suggest that these effects are not species-specific, but a general feature of heterogeneous myocardial systems (Markhasin et al., 2012).

The change in contractile activity of individual muscle elements during their mechanical interaction in the duplex setting can be revealed also upon duplex uncoupling, as either an overshoot or undershoot of individual element isometric peak forces is seen (see Fig. 5, phase 4). In most of the duplex experiments, the individual element responses are opposite in direction, reflecting the respective "gain" or "loss" in their individual element contractility (Fig. 5, (Markhasin et al., 2012)). Less frequently, we observed synergistic overshoots (or undershoots) in both muscles after termination of duplex interactions (Solovyova et al., 2006). A systematic analysis of individual muscle behaviours in theoretical and experimental SFR_{IM} settings showed a significant correlation between deformation patterns and individual contractility changes in interacting duplex elements (Markhasin et al., 2012).

Along with the mechanical manifestations of SFR_{IM}, *in silico* experiments revealed slowly developing beat-by-beat changes both in the AP and the Ca^{2+} transient shape and duration in cardiomyocytes of interacting virtual muscles (Markhasin et al., 2012). In virtual duplexes, individual SFR_{IM} of interacting VM follows the respective gradual gain (or loss) in SR Ca^{2+} loading, which depends on dynamic deformations of the muscle during contraction (Markhasin et al., 2012). In agreement with *in silico* predictions, we observed beat-by-beat changes in AP shape and duration in interacting BM preparations in biological duplexes (Fig. 5), and pronounced changes in intracellular Ca^{2+} transients in BM of hybrid duplexes (Markhasin et al., 2012), constituting what could be called a contact-free redistribution of intracellular Ca^{2+} content.

4. Extension of the duplex model: one-dimensional models of myocardial tissue

Given the relevance of dimensionality for cardiac modelling (Garny et al., 2005), we developed multi-cellular cardiac tissue models, beginning with a one-dimensional (1D) chain consisting of several virtual cells, mechanically connected *in series* (Solovyova et al., 2006). Such discreet models allow us to simulate gradual changes in spatio-temporal properties of cardiac tissue and to study effects of activation sequences with prescribed timing of regional stimulation mimicking propagation of electrical excitation in the chain. The model does not, at this stage, specifically differentiate between intra- (e.g. titin) and extracellular (e.g. collagen) viscoelasticities.

The most substantial limitation of this model is that electrotonic interactions between cardiomyocytes are not accounted for. Recently, we developed a continuous 1D mathematical model of myocardial tissue, as a muscle strand formed of mechanically *and* electrically interacting cardiac muscle elements (Katsnelson et al., 2014). Any cardiomyocyte in the model is considered as a functional point of myocardial tissue, which forms a continuous 1D medium. Inherent electrical and mechanical properties of the cells (cellular model parameters) in the tissue model could be either identical (homogeneous model) or vary (inhomogeneous model). For homogeneous models, the nearest experimental equivalent could be systems such as linearly-structured cardiomyocyte cultures, or cells grown 'on a thread' (Camelliti et al., 2006; Proulx et al., 2011). Inhomogeneous 1D tissue models may be seen to mimic a range of interactions in myocardial 'fibres' running in an orderly arranged set of directions inside the intact ventricle (Markhasin and Solovyova, 2005), while experimental models such as isolated trabeculae subjected to regional perfusion that causes

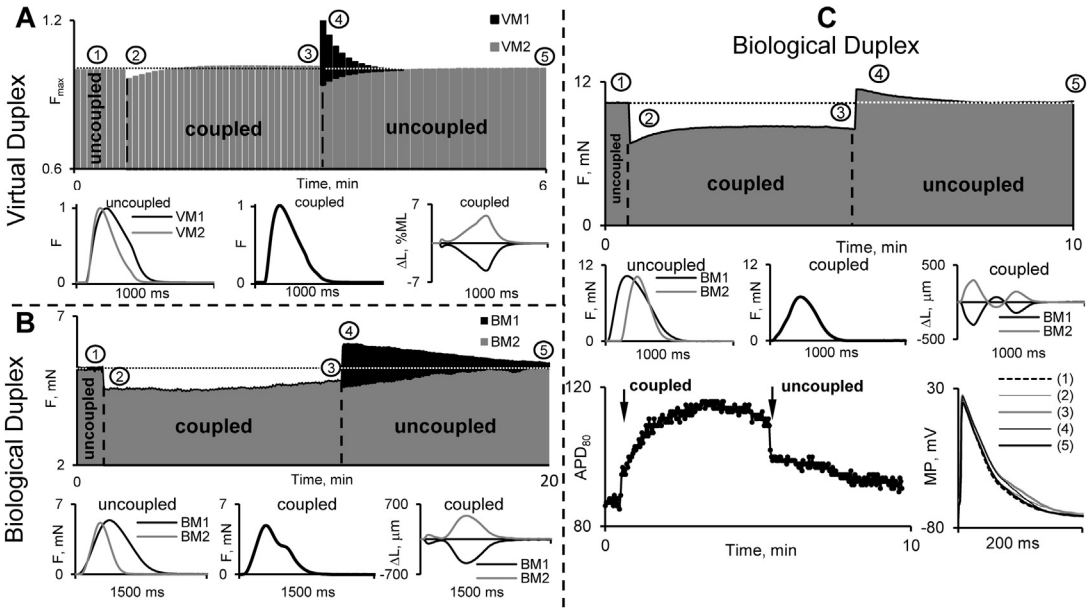

Fig. 5. Intra-myocardial slow force response (SFR_{IM}) registered in virtual (A) and biological (B, C) myocardial duplexes. A–C, top: Example SFR_{IM} in heterogeneous duplexes, comprising of a slow and fast muscle with a stimulation delay of the fast muscle. Phases (1)–(5) show transitions of the peak force developed by each muscle in isolation, once coupled *in series*, and after duplex disconnection. Next rows in A–C show the time course of steady-state isometric forces, developed by the muscles before coupling with a signal time shift equal to the stimulation delays used after muscle coupling (left); the time course of force development by the *in series* coupled muscles (middle); and length changes in the two muscles (right) during the duplex contractions. In the virtual duplex, force is normalized to the peak force at phase 1, shortening is expressed as the % of initial muscle length. Biological duplexes comprise two papillary muscles from rabbit (B) and rat (C) right ventricle, contained at either 25 °C or 30 °C, with matching individual peak forces at phase 1. In panel C, mechanical activity in the first duplex element was measured simultaneously with cellular MPs. Changes in AP duration at 80% repolarization (APD_{80}), measured in one and the same cardiomyocyte of this muscle during the force transitions, and superposition of AP during muscle contractions at every phases (1)–(5) are shown. Experimental data are from Markhasin et al. (2012), with permission.

local alterations in contractility (ter Keurs et al., 2006) could be used for model validation.

Each cardiomyocyte in the 1D tissue model has its own local, dynamically changing shape and mechanical environment within the tissue during the contractile cycle. As in native tissue, the electrical wave of excitation propagates along a dynamically deformable medium. Our 1D model accounts for both micro- and macrocircuits of the electro-mechanical and mechano-electric interactions in heart tissue. Each cell is described by our EO model of cellular electro-mechanics, excitation propagation is governed by a reaction-diffusion equation, and macromechanics at each point is defined, accounting for integrated cellular mechanics along the entire fibre.

Utilizing this 1D tissue model, we reconfirmed qualitative predictions made in discreet muscle-chains. First, we showed in homogeneous 1D models, formed of identical virtual cells, that the excitation sequence gives rise to regional deformations which cause functional heterogeneity of individual cardiomyocytes ((Katsnelson et al., 2014), see also Fig. S5 in the supplement). Remarkably, gradients in APD and SR Ca^{2+} loading along the fibre were produced by running an orderly excitation sequence along identical myocytes, and these increased significantly upon reduction in the velocity of propagation, as this increases heterogeneity in cell dynamic deformations within the fibre (Katsnelson et al., 2014).

In heterogeneous 1D tissue models, presented here for the first time, we utilized our recently-developed cellular models of sub-endocardial (ENDO) and sub-epicardial (EPI) cardiomyocytes (Vasilyeva and Solovyova, 2012). One third of the tissue strand consisted of the ENDO models, one third of EPI models and the middle third was formed of cells with gradually varying parameters between the ENDO and EPI cells (Fig. 6). In this inherently heterogeneous 1D model, we found that excitation waves directed from ENDO to EPI regions reduce or eliminate DR, supporting an inverted repolarization wave (travelling in the opposite direction to that of depolarization; Fig. 6), as seen in normal myocardium (Franz et al., 1987). In this case, electro-mechanical coupling of the cells did not decrease force production in the tissue (as compared to the force produced in any of its segments in isolation). Inverted excitation waves, directed from EPI to ENDO regions, caused a significant reduction in tissue force development, and an increase in the DR in the tissue, accompanied by an increase in the amplitudes of regional deformation and a respective increase in the heterogeneity of useful work performed by different regions within the 1D tissue (Fig. 6). This re-emphasises the point that externally uniform performance of the heart builds upon, and necessarily requires, built-in functional heterogeneities at the cellular level.

5. Duplex models applied to the study of cardiac pathology

The muscle duplex approach allows one to study contributions of mechanical heterogeneity in myocardial tissue to normal and disturbed electrophysiological function, using an integrated theoretical and experimental approach. Effects of heterogeneous muscle segment interactions have also been studied in single-strand muscle preparations by ter Keurs' and Miura's groups (see, for example (Miura et al., 2010; ter Keurs et al., 2006)). In these experiments, single trabeculae were exposed to local perfusion to render central parts of the muscle hypocontractile. Lessons learnt from these studies are relevant to the *in series* duplex concept, as discussed below.

We studied effects of mechanical interactions using *in series* virtual duplexes composed of muscle segments with varying Ca^{2+} contents, from normal to severe Ca^{2+} overload, induced by various levels of reduction in Na^{+}–K^{+} pump activity in the cellular EO model (Sulman et al., 2008). Severe Ca^{2+} overload in cells with

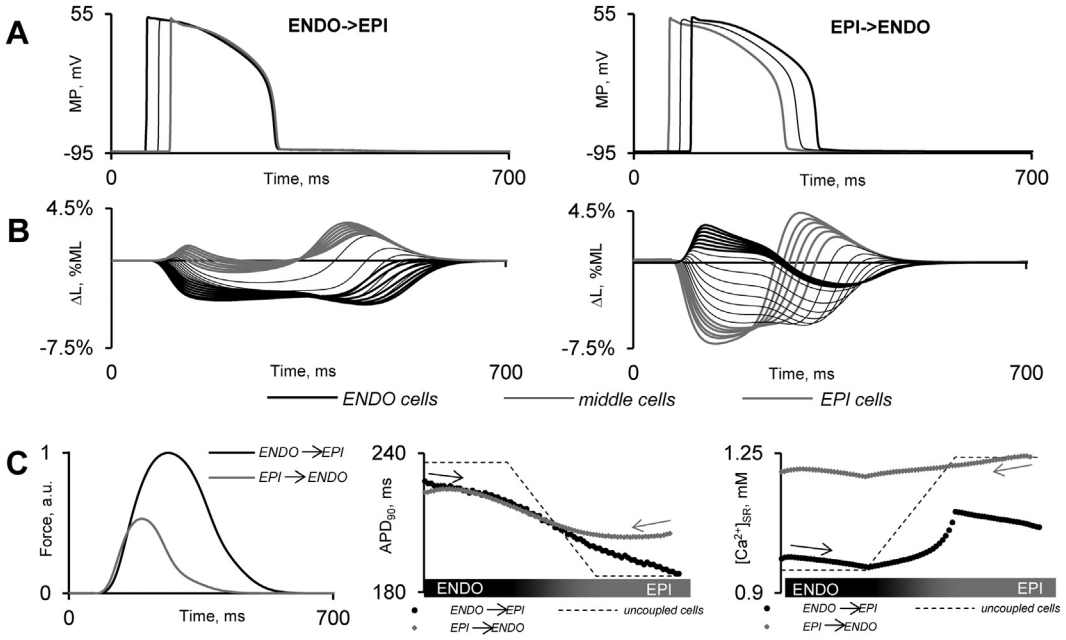

Fig. 6. Effects of the activation sequence on electrical and mechanical activity in a heterogeneous 1D virtual tissue of 20 mm length. Depolarization waves were initiated at either edge of the 1D strip, at a pacing rate of 1 Hz. Excitation spread, with a velocity of ~0.6 m/s, from sub-endocardial (ENDO, black) towards sub-epicardial cells (EPI, grey), or in the opposite direction (EPI → ENDO). Panels A and B show the time course of cellular membrane potential (MP) and deformation (ΔL, % of the initial muscle length ML) in different regions along the tissue, in panel A also illustrating the activation sequence. Panel C shows the time course of the external force, developed by the 1D model, in corresponding externally isometric twitches (normalized to the peak of duplex contractions during any of the activation sequences). The centre and right traces in panel C show gradients of APD at 90% repolarization (APD_{90}) and diastolic SR Ca^{2+} loading ($[Ca^{2+}]_{SR}$), developed in the 1D model over 30 beats depending of the excitation sequence (arrows indicate direction of the depolarization wave). The dashed lines show corresponding values observed in uncoupled virtual cells.

strongly decreased Na^+–K^+ pump activity ($K_{m,Na}$ raised to 165% of normal, causing intracellular Na^+ accumulation, followed by Ca^{2+} overload) led to spontaneous Ca^{2+} releases from the SR with subsequent electrical activation of the cell, similar to earlier modelling-based observations on spontaneous SR Ca^{2+} release as a possible contributor to ischaemic arrhythmogenesis (Ch'en et al., 1998). These 'ectopic' AP developed independently of the mechanical environment of the cell. In cardiomyocytes with less severe reduction of Na^+–K^+ pump activity, emergence of spontaneous activity was sensitive to the mechanical environment. In particular, reductions in mechanical pre- or afterload gave rise to spontaneous AP generation in the pathologically disturbed duplex element (Sulman et al., 2008). These model predictions were experimentally verified on papillary muscles from guinea pig right ventricle, exposed to the Na^+–K^+ pump inhibitor ouabain (Katsnelson et al., 2011). The results suggest that ectopic activity in pathological foci and border zone tissue can be promoted by their mechanical interactions with normal or less severely affected myocardium (Sulman et al., 2008).

We tried to assess this hypothesis in a 1D tissue model, formed of 90% normal tissue (N-cardiomyocytes), coupled to 10% of sub-critical (SC; $K_{m,Na}$ 157% of normal (Sulman et al., 2008)) cells at one edge of the strand. These SC-cells do not develop spontaneous activation at reference isometric conditions in isolation. We studied 1D strand contractions paced at 1 Hz from the N-cell end in externally isometric mode (not shown). Within about 300 beats after cell coupling, the virtual tissue developed bursts of extra-systolic activity. This spontaneous activity in the SC-zone arose between regular waves of excitation, and it was preceded by spontaneous Ca^{2+} releases, which induced either sub-threshold membrane depolarizations, or extra-APs in SC-cells. Extra APs could induce an ectopic focus, which spread retrogradely, invading the normal tissue. In this model, the trigger activity in the SC-zone emerged after hundreds of cyclic deformations, that shifted the 'anyway present' Ca^{2+} accumulation (as a result of Na^+–K^+ pump inhibition) beyond the threshold for spontaneous Ca^{2+} release from the SR. Further muscle activity comprised of multiple successive intervals of regular contractions, interspersed by bursts extrasystoles.

Simulations in these 1D tissue models extend duplex predictions by additionally accounting for electrotonic cell coupling, a pre-condition for the spread of ectopic waves. Model simulations suggest that the mechanical interactions between cells create, via mechanisms of mechano-electric coupling, a substrate for arrhythmia in heterogeneous tissue that contains an otherwise benign area of Ca^{2+}-overloaded. Model analysis suggests that dependency of Ca-TnC binding kinetics on the force-generating cross-bridge concentration (a key cooperativity mechanism) plays an important role in triggering spontaneous activity in myocardial tissue whose regional heterogeneity is facilitated by pathology.

6. Discussion

Using six configurations of experimental and virtual cardiac muscle duplexes, we found a number of effects characteristic for mechanically-interacting, but not necessarily touching, muscle segments in heterogeneous myocardium. Several effects were first predicted in "dry" numerical experiments on virtual duplexes, and then confirmed in targeted "wet" physiological studies, using biological and/or hybrid duplex configurations. Analysis of intracellular processes in VM allowed us to identify possible mechanisms, underlying the observed macroscopic effects. Similarities between duplex activity predicted theoretically and observed experimentally allowed us to identify some of the mechanisms that appear to govern the activity in interacting cardiac muscle segments. Advantages of the duplex approach become obvious when considering some of the effects, unravelled with its help.

In parallel duplexes we found that intrinsically different force–velocity curves of duplex elements approach each other, if activation of the faster element occurs with a suitable delay compared to the slow element. This also avoids potentially arrhythmogenic increases in DR, which thanks to the sequential activation is reduced below inherent net differences in APD of the muscles in isolation. We call this, and a number of related effects where sequential muscle activation improves their combined functional state a *tuning effect*, to highlight the functional fine-tuning that occurs between elements, as they adjust their mechano-electric activity profiles to one another in the duplex.

Opposite effects, force–velocity divergence and increased DR, were observed in all *in parallel* duplex configurations if the normal activation sequence was inverted.

Interestingly, a common feature of *in parallel* duplexes was that duplex force–velocity behaviour showed little sensitivity to modulation of individual element F–V curves caused by alterations in the sequence and time delay of muscle activation, as 'losses' in one element were usually compensated by 'gains' in the other (see Fig. 4 and (Markhasin et al., 2011)). If present *in situ*, this could be an important autoregulatory compensation response. We call this *contractility conservation*, a system's property that is ensured by reciprocal changes of the mechanical characteristics of individual interacting muscle elements. This phenomenon is compatible with the high stability and adaptive reserve of normal heart muscle. However, as the force–velocity curve of the heart is widely used to characterise muscle contractility, a note of caution is warranted: overtly normal function of a ventricle can involve (and, hence, hide) sub-critical local heterogeneities and dysfunctions that, as they increase in size or severity, may contribute to myocardial pathology.

The convergence of force–velocity and force–work curves, observed in heterogeneous parallel duplexes may represent a mechanism involved in interactions between ENDO and EPI layers of the LV. It has been shown that isolated cardiomyocytes from ENDO layers contract and relax more slowly than EPI cells, and that the duration of AP and intracellular Ca^{2+} transients is longer in ENDO than in EPI cells (Cordeiro et al., 2004; Laurita et al., 2003; Wan et al., 2003). As ENDO cells are excited earlier than EPI (Sengupta et al., 2006b), parallel duplexes consisting of a slow and a fast contracting element may serve as a simple qualitative model of interactions between different transmural layers of the ventricular wall.

Disturbances in the normal activation sequence can give rise to divergence of force–velocity behaviour and work in parallel transmural layers of LV. This includes pathological settings, as well as therapeutic approaches such as epicardial pacing, widely used for cardiac resynchronization therapy (CRT) (Kirn et al., 2008; Yu et al., 2005). Duplex behaviour would predict that the force–velocity and work curves in one region should increase (reflecting a rise in contractility), potentially associated with a rise in regional oxygen consumption. In the CRT setting, if a ventricle already suffered from compromised vascular supply, such increased demand could further raise the mismatch between supply and demand.

The mechanisms underlying interaction effects in muscle duplexes can be theoretically explored in virtual duplex models. These predict opposite changes in cellular concentration of Ca–TnC complexes in interacting muscle elements, causing their force–velocity curves to move in opposite directions. Mechano-dependent modulation in the kinetics of intracellular Ca^{2+} handling, whether caused by TnC buffer effects (Markhasin et al., 2012; ter keurs, 2012), or involving mechanical modulation of sarcolemmal and/or SR ion channels (Iribe et al., 2009; Jie et al., 2010; Morad et al., 2005; Sachs, 1986), will consequently affect AP shape and duration, and thus contribute to a decrease or increase in DR. Again, this will strongly depend on the activation sequence (see Fig. S4 in the supplement).

A shift in individual force–velocity curves in heterogeneous duplexes occurs due to mechanical load redistributions between interacting muscles during their contractile interaction. This means that the inotropic state of muscle segments depends *not only* on the characteristic velocity of cross-bridge cycling (determined by the myosin isoforms in myofilaments), but also on the velocity and extent of formation of Ca–TnC complexes. This cooperativity is a direct consequence of the fact that the affinity of TnC for Ca^{2+} also depends on mechanical conditions, which can vary in pathology (Apstein et al., 1987; Grossman et al., 1977; Ilkovski, 2008; Pinto et al., 2009; Schober et al., 2012).

So, is all this heterogeneity a bad thing that the heart somehow 'has to cope with'? In 1989, A. Katz and P. Katz published a keystone article on cardiac "*homogeneity out of heterogeneity*" (Katz and Katz, 1989). They illuminated the frequently overlooked fact that intrinsic heterogeneities in the mechanical activity of different ventricular areas leads to an optimal functional uniformity of their collective performance during ventricular contractions. They suggested that cells in each myocardial region are adapted to the mechanical conditions which they encounter. Such adaptation may result from gene regulatory effects on expression of isoforms of contractile and membrane proteins in different ventricular layers (Katz and Katz, 1989), which explains how inverted activation sequences (e.g. in CRT) may remodel cells in the myocardium. The authors thus identify regional heterogeneity to be a *prerequisite* for effective myocardial function at the organ level. Their hypothesis, therefore, is focused on the idea that heterogeneous regions collectively allow homogeneous function.

The data derived from the duplex experiments suggest an even more complex picture, namely one in which myocardial heterogeneity is not only present and prerequisite, but also dynamically changing on a beat-by-beat time scale. We propose, therefore, that the interrelation of local heterogeneity and global homogeneity constitutes a bi-directional setting. Our results demonstrate significant and dynamic adjustments of regional electrical and mechanical functions to changing environmentally conditions. These have the potential of optimising and adapting regional function to overall cardiac demand. Moreover, our results suggest a key role of *activation timing* in these regional interactions, embedded in two 'vectorial' characteristics: time sequence (activation order) and time interval (activation delays). This may shed new light on the relevance of interventions with dromotropic effects, which at present could be undervalued contributors to cardiac (auto) regulation.

In series connection of duplex elements influences the mechanical and electrical function of the whole duplex and of each element (Figs. 3 and 5). As shown in Fig. 3, activation delays of the fast muscle element within a certain range do not reduce the mechanical force, and eliminate potentially arrhythmogenic DR between elements (Markhasin et al., 2004; Solovyova et al., 2003). In contrast, any activation delay of the slow muscle element reduces peak force development and increases DR. We view these *in series* heterogeneous duplexes as mimicking regional interactions in the LV of the intact heart, where the later-activated (e.g. basal) segments contract faster than *in series* connected apical segments that are activated earlier (Sengupta et al., 2006a). Specific features of *in series* duplex performance, depending on activation sequence, were found to be qualitatively similar in all duplex configurations (Markhasin et al., 2003). In all cases, interactions between elements led to slowly developing beat-by-beat modulation of the functional state of individual duplex elements. This can be referred to as 'contact-free redistribution' of Ca^{2+}, as one element gains, while the other sheds, intracellular Ca^{2+} – in spite of not being placed in a

common perfusion space. We refer to associated changes in contractility as an *intra-myocardial slow force response* (SFR_{IM}) (Markhasin et al., 2012). We show that the individual elements alter their contractility, usually in opposite directions, similar to what was observed in parallel duplexes. Importantly, experimental data also show beat-by-beat changes in cellular AP generation and Ca^{2+} transients, along with slow changes in the mechanical activity of interacting muscles, all of which are in good agreement with model predictions (see Fig. 5; (Markhasin et al., 2012)).

Phenomena from *in series* duplexes also highlight the important role of time in the spatio-temporal organization of cardiomyocytes in the ventricular wall. To investigate this issue further, we studied mechanical interactions between several virtual muscle elements in extended mechanically-linked chains that allow one to explore effects of the activation sequence on spatial distribution of regional electrical and mechanical properties over longer distances, and with a more varied composition of elements (Solovyova et al., 2006). These models were initially chained together mechanically only, but have since been developed into a 1D continuum tissue model, which accounts for both electrical and mechanical interaction between cardiomyocytes (Fig. 6, Fig. S5). Triggered activation propagates along these 1D virtual tissue strands, creating graded heterogeneities in regional functional properties of interacting cardiomyocytes (see Fig. S5, and (Solovyova et al., 2006)). A field of dynamic deformations, produced by the mechanical interactions between cardiomyocytes activated at slightly different time points, gives rise to gradients of AP and intracellular Ca^{2+} transient shape and duration. This can be associated with emergence of a remarkable gradient in SR Ca^{2+} loading along the tissue (Fig. S5, (Solovyova et al., 2006)). Effects of regional deformation on cellular functional properties suggest that mechano-electric feedback mechanisms contribute to these phenomena. As a result, intrinsically homogeneous tissue becomes functionally heterogeneous, due to sequential activation (propagating excitation) and mechanical interaction (across the whole tissue model), with potentially interesting implications for force production at the tissue or organ level. This may be attenuated depending on the velocity of depolarization wave propagation, as opposed to a setting with simultaneous excitation (see Fig. S5), where the DR is the higher, the lower the velocity of propagation (for homogeneous models; not shown).

One question that arises from the above is: what spatio-temporal organisation of intrinsic electro-mechanical properties of cardiomyocytes within the tissue may optimise myocardial function (i.e. what is characteristic for "well-organized heterogeneity")? Following predictions from duplex studies, we composed a 1D strand of tissue with inherently heterogeneous segments, where the earlier activated segments produce force more slowly than the later activated ones (Fig. 6, and (Solovyova et al., 2006)). In this 1D model, using recently developed ENDO and EPI cell descriptions (Vasilyeva and Solovyova, 2012), overall force production is less sensitive to changes in the velocity of excitation, compared to homogenous tissue strands. In addition, in heterogeneous 1D models, intrinsic DR is reduced as a consequence of the 'normal' spread of excitation (Fig. 6). While simple, this model reflects key principles of the optimal coordination of regional (transmural and/or longitudinal) properties of cardiomyocytes in the intact ventricle. In general, earlier-activated regions have longer APD and slower contractions, than cells from regions that are activated with time delays. This cellular functional heterogeneity intrinsically matches excitation timing, arising from wave propagation in the ventricle, and so the combined effect of temporal and spatial heterogeneities serves to optimise overall electrical and mechanical function of the heart.

In contrast, 'inverted' activation sequences in muscle duplexes, mechanically linked chains, and 1D continuous tissue models (e.g. Fig. 6) show that disturbances in electrical excitation timing may increase electrical heterogeneity of cardiomyocytes and even contribute to generation of arrhythmogenic substrates (Section 4). These modelling results gain further relevance from experimental studies, showing that 'retrograde' pacing of the LV may cause ventricular electrical remodelling (Jeyaraj et al., 2007; Libbus and Rosenbaum, 2003). David Rosenbaum's research in particular showed that this electrical remodelling strongly correlates with regional deformation patterns, induced by pacing, and suggested that cardiac mechano-electric feedback may play a key role in cardiac memory (Jeyaraj et al., 2007).

Myocardial heterogeneity can increase pre-existing pathological imbalances (Bolli and Marban, 1999; Donker et al., 2005; Haluska et al., 2003; Ikeda et al., 2008; Plazak et al., 2002). In our duplex and tissue models with local Ca^{2+} overload (Section 5 and (Sulman et al., 2008)), we found that regional mechanical interactions may amplify pathological heterogeneities in myocardial tissue function, and facilitate emergence of ectopic activity. It is noteworthy, that even a relatively small number of sub-critical cells may be able to initiate ectopy and rhythm disturbances, affecting the entire tissue. This is in keeping with earlier predictions by Winslow et al. (Winslow et al., 1995), who showed that Na^{+}-overloaded cells, forming a sphere with a 10-cell radius only, would be sufficient to trigger propagating waves of ectopic excitation in a block of $128 \times 128 \times 32$ atrial cell models. These small volumes – in terms of real cells, the critical volume would be less than 1 mm^3 – are well below the resolution of standard medical imaging modalities, contributing to their potential clinical significance.

As we mentioned in Section 4, effects of cardiac mechanical heterogeneity on arrhythmogenesis have also been studied in native tissue strands by ter Keurs, Miura, and colleagues (see, for example (Miura et al., 2010; ter Keurs et al., 2006)). Their method builds on creating in series heterogeneities by local perfusion of a single trabeculae with solutions that perturb regional mechanics, i.e. a setting we explore with *in series* duplexes and/or 1D virtual tissue strands. A key benefit of their method is that interactions between coupled muscle segments are not only mechano-electric but that they also include ion fluxes between regions in an organotypic heterocellular setting. Such a complete set of physiologically relevant conditions has advantages (closer to *in situ*) and disadvantages (less control over individual forms of interaction). In addition, single trabeculae cannot be used to explore *in parallel* coupling effects). So, while the duplex approach allows one to detect and compare, for example, the roles of *in parallel* and *in series* mechanical interaction for local adjustment in Ca^{2+} kinetics and sarcomere mechanics, the heterogeneous trabeculae model allows one to assess the relevance of such adjustments for pathophysiological behaviour at a more holistic level. Published data, obtained with both methods, agree with one another, in that they highlight the role of mechano-dependent cooperative mechanisms underlying a range of effects of mechanical interactions between heterogeneous cardiomyocytes, including contributions to arrhythmogenesis. Therefore, the methods should be considered as complementary to one another.

Thus, the duplex approach has allowed us to explore several aspects of the causal chain underlying effects of electro-mechanical and mechano-electric coupling in heterogeneous myocardial systems. This chain, which gives rise to functional adjustments in cell activity to tissue/organ demand, can be simplified as consisting of the following intracellular sequence: electro-mechanical coupling → mechano-dependent Ca^{2+} kinetics → Ca^{2+} dependent ionic currents → Ca^{2+} loading and membrane potential modulations → mechano-electric coupling, and so on. One central link in this causal chain is cooperativity of Ca^{2+} activation of myofilaments, which gives rise to mechano-dependent kinetics of Ca-TnC

complexes, and provides for mechano-dependent intracellular Ca^{2+} kinetics. Of course, this feedback loop is far from comprehensive, as it will also include contributions from several other directly mechano-dependent mechanisms, such as stretch-activated ion channels in the sarcolemma and intracellular membrane systems including Ca^{2+} stores (Friedrich et al., 2012; Hu and Sachs, 1997; Iribe et al., 2009; Kohl et al., 2006; Morad et al., 2005), mechano-dependent phosphorylation of regulatory proteins and activation of kinases and mediators (Berridge, 2003; Kohl and Noble, 2008), or effects mediated by cardiac non-muscle cells (Kiseleva et al., 1996; Kohl et al., 1994; Kohl and Gourdie, 2014). Their inclusion will help to explore further contributors to normal and disturbed heart function (Jie et al., 2010; Keldermann et al., 2010; Li et al., 2004; Nash and Panfilov, 2004; Trayanova et al., 2004).

7. Conclusions and outlook

From a methodological point of view, the cardiac muscle duplex is a useful tool in several regards. The combined implementation of theoretical and experimental duplex configurations allows one to interlink 'wet' data generation for 'dry' model development and 'dry' model prediction to 'wet' experimental validation – a key feature of systematic bio-research (Hunter et al., 2001; Quinn and Kohl, 2013). Similarities between results obtained in 'dry' and 'wet' experiments, but even more so discrepancies, are drivers of conceptual insight. For example, the mathematical models utilized in our heterogeneous duplexes were developed and verified against experimental data obtained without accounting for myocardial heterogeneity; model simulations in new environmental conditions thus served as a tool for discovery of new phenomena in the myocardium.

We believe that a hybrid systems research approach, allowing for real-time interactions between a biological preparation and its computational model equivalent, is of particular relevance for pathophysiological applications. In the hybrid duplex approach, a biological preparation is controlled by input signals driven by a computational model, whose output in turn depends on the signals received from the real biological muscle (Protsenko et al., 2005). As this bilateral signal exchange occurs in real time, hybrid duplexes allow one to explore responses of one and the same biological preparation upon interactions with various virtual cell partners, and using both *in series* and *in parallel* interaction configurations. As for cardiac mechanics, to our knowledge, computer-model driven techniques for the bi-directional and dynamic mechanical *interaction* with a biological multicellular preparation have been implemented for the first time in our studies (Protsenko et al., 2005). Mechanical interaction studies on cardiac tissue are particularly useful if combined with measurements of intracellular Ca^{2+} dynamics and cellular membrane potentials (see Fig. 5, and (Markhasin et al., 2012)).

Building on the importance of local heterogeneity for global homogeneity, presaged by Katz & Katz, dynamic cellular adaptation to the prevailing mechanical conditions is needed to allow cardiomyocyte heterogeneity to match their specific mechanical environments. We can only speculate how the relevant cellular heterogeneities develop and adjust in ontogenesis, during changing demands (physical activity, pregnancy), and in pathogenesis. As mentioned above, this is likely to include effects of regionally varying mechanical stress/strain behaviour on the synthesis of distinct isoforms of contractile and membrane proteins in cells, which may make cells more 'suitable' for their location in the myocardial wall (Biesmans et al., 2011; Jalil et al., 1988; Ver Heyen et al., 2001).

Beyond the bi-directional nature of cross-talk between local hetero- and global homogeneity, the perhaps most relevant result that emerged from our bi-directional duplex studies is an appreciation of the role that the coordination of activation sequence and intrinsic cellular heterogeneities play in allowing externally homogeneous heart function. The activation sequence contributes to beat-by-beat tuning of cardiomyocyte properties, via functional effects of electro-mechanical and mechano-electric coupling. Modulation of cyclic mechanical activity and intracellular Ca^{2+} dynamics in coupled cells may hold a key to the recruitment of mechano-transcriptional coupling (Bers and Guo, 2005; Bers, 2011; Buyandelger et al., 2011), which is mechano- and Ca^{2+}-dependent (Berridge, 2003; Sadoshima et al., 1992). These epigenetic processes may manifest themselves over medium- to long-term periods and contribute to physiological remodelling of the heart. Similar mechanisms may be at play in ageing or during pathologies, resulting in discoordination of ventricular function (Kirn et al., 2008; Quinn, 2014). Changes in ventricular geometry (such as hypertrophy or dilatation) induce changes in mechanical stress-strain fields in the myocardium, which are thus far insufficiently explored. Overlaid by disturbances in the activation sequence (whether from rhythm disturbances, caused by CRT, or during ventricular unloading by mechanical assist devices) will further complicate the picture. Perhaps, the difference between physiological and pathological tissue remodelling is the question of whether or not it gives rise to a sustained mismatch between activation sequence and cardiomyocyte functional properties.

All this complexity highlights, in our view, the need for simple models that are just about complex enough for the study of myocardial heterogeneity, and that have clear translational potential.

Editors' note

Please see also related communications in this issue by Iribe et al. (2014) and Livneh et al. (2014).

Acknowledgements

The authors gratefully acknowledge the kind help of Mrs. Katherine Fletcher with careful revision of the text, and research support from the Ural Branch of the RAS (12-M-14-2009, 12-П-4-1067), Programme #1 of Basic Research of the Presidium of RAS, Ural Federal University (No.211 Decree of the Government of RF of 16th March 2013), and the Russian Foundation for Basic Research (14-01-31134, 14-01-00885). PK is a Senior Research Fellow of the British Heart Foundation and supported by a European Research Council Advanced Grant (CardioNect).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.pbiomolbio.2014.07.010.

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